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Betta siamorientalis, a new species of bubble-nest building fighting fish (Teleostei: Osphronemidae) from eastern Thailand

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> Abstract

Betta siamorientalis sp. n., a new species of fighting fish belonging to the *Betta splendens* group, inhabiting four provinces in the eastern Thailand, is described. The combination of slender body shape with reddish vertical bars on the black opercle and an apparent black body distinguishes the new species from other congeners especially *B. imbellis*. The blacker appearance relative to *B. imbellis* is due to a higher proportion of black body area relative to that occupied by blue/green iridescence of the scales. Based on morphological similarity and fin colour pattern and vertical bar colour on the opercle, the new species appears to be more closely related to *B. imbellis* and *B. splendens* than other members of the *B. splendens* group.

> Key words

Betta siamorientalis sp. n., Black imbellis, Bubble-nesting fighting fish, Thailand.

Introduction

Based on behaviourial and morphological characters and details of head and body patterns, Witte & Schmidt (1992) have classified betta fighting fishes into 15 species groups (see also Tan & Ng, 2005), one of which is the *Betta splendens* group. The term species group is used here for an assemblage of species sharing diagnostic characters. Members of the *B. splendens* group are bubble-nest building bettas with the following characters: small head, often brightly coloured body, iris of eye with iridescent green or blue patches, body slender, head with parallel opercles when viewed dorsally, red or brown anal and caudal fin rays contrasting with iridescent interradial membrane (especially in male) (Witte & Schmidt, 1992). These members are *B. splendens* Regan, 1910; *B. smaragdina* Ladiges, 1972; *B. imbellis* Ladiges,1975; *B. stiktos* Tan & Ng, 2005; and the newly identified *B. mahachaiensis* Kowasupat, Panijpan, Ruenwongsa & Sriwattanarothai, 2012). It is well recognised that the native habitats for wild *B. smaragdina* are in the northeast and Laos PDR, those for *B. imbellis* are in south of Thailand extending to peninsular Malaysia. *Betta splendens* is distributed more or less countrywide from the north to central to the upper southern peninsula and also from east to west of Thailand and beyond. *Betta mahachaiensis* has been found confined to the Samut Sakhon province, west of Bangkok (Kowasupat *et al.*,2012). Since recorded history,

Thais have reared these fighting fish and bred them, especially, *B. splendens*, for both fighting and ornamental purposes (Smith, 1927, 1945; MONVISES *et al.*, 2009). However, there are only a few reports on the distribution of the bubble-nest building fighting fish in Thailand or nearby countries. For example, LERTPANICH & ARANYAVALAI (2007) had identified the fish from the eastern Thailand all as *B. splendens* based mainly on the presence of reddish bars on the opercle. Our ongoing extensive fieldwork since 2007 on *Betta* distribution all over Thailand has now covered sixty-seven provinces (as of 2012) of the total seventy-seven including all seven provinces in eastern Thailand and some bordering Cambodia and Laos PDR. It has been noted by us and others that the betta fighting fish in the Chachoengsao province and some other eastern provinces possess different characteristics to other known members of the *B. splendens* group (authors′ personal communication). Locals say such unique characteristics have been known for generations, but the red bars on the opercle have persuaded them to believe the fish to be *B. splendens*. This fish has been called black imbellis by those familiar with it due to its black opercle and apparently black body. This black imbellis has a general appearance quite similar to the fish caught from Cambodia in Seam Reap by KÜHNE (2008), who named it *Betta* cf. *imbellis*, and similar to the *Betta* sp. in the Priay Khmang village by LINKE (2009). SCHÄFER (2009) also received from Vietnam some traded fish and identified it as *Betta* cf. *stiktos* based on preserved specimens of the fish for comparison. However, upon later comparative work with live wild-caught specimens from the type-locality, he then changed the name to *Betta* cf. *imbellis* and called it Vietnam black emphasising the paucity of blue/green iridescence on the black body.

Upon in-depth analysis, our data suggested that the fish was a new species, at least those found in four provinces of eastern Thailand: Chachoengsao, Sa Kaeo, Prachin Buri and Chonburi (near border of Chachoengsao) and also at one site in Cambodia across the border from Sa Kaeo (KOWASUPAT & PANIJPAN, 2012; LINKE 2012). The aim of this paper is to formally describe this so-called black imbellis.

Materials and methods

Fighting fish specimens were preserved in 70% ethanol for long-term storage. The left side of all specimens was used for morphometric measurements point to point using digital Vernier caliper readings to the nearest 0.1 mm according to the method of SCHINDLER & SCHMIDT (2006) and TAN & NG (2005), both modified from WITTE & SCHMIDT (1992). Ratios are given in percent standard length or percent head length. The terminology and general format of description is based on WITTE & SCHMIDT (1992), TAN & KOTTELAT (1998) and TAN & NG (2005). Meristic counts were carried out as described by WITTE & SCHMIDT (1992) except for the number of predorsal scales, which were counted continuously following NG & KOTTELAT (1994). The phylogenetic species concept is used in this study (CRACRAFT, 1989; see TAN & NG, 2005).

All statistical analyses were performed using SPSS Version 20. To avoid undesirable effects of using ratios in statistical analyses, the logarithms of these ratios (LGRAT) were used (ATCHLEY & ANDERSON, 1978). The LGRATs of each trait of each species were tested for normality using the Shapiro-Wilk test. For non-normal traits, the Kruskal-Wallis one-way ANOVA together with Mann-Whitney U test were used to compare means. Otherwise, the Levene′s test was used to test for homogeneity of variances. If the variances were equal, the one-way ANOVA together with the Tukey post-hoc test were used to compare means. Conversely, the Welch one-way ANOVA and the Games-Howell post-hoc test were used. Significant level was adjusted by the Bonferroni correction.

Specimens examined have been deposited at the Thailand Natural History Museum, Pathum Thani (THNHM) and also kept in the authors' own collection for the Thailand Betta Project (TBP). Abbreviations used are SL – standard length, HL – head length, and TL – total length.

Comparative material

The following materials were compared for morphometric and meristic characteristics as well as general appearance and body shape.

Betta imbellis caught from thirteen provinces of southern Thailand, one province of Malaysia (type locality), and one province of Vietnam
(THNHM-F-01557 – 61, THNHM-F-01564 – 6, THNHM-F-01569, THNHM-F-01571, THNHM-F-01573, THNHM-F-01575 – 7, THNHM-F-01579, THNHM-F-01582, THNHM-F-01585 – 7, THNHM-F-01596 – 600, THNHM-F-01829, THNHM-F-01841)

Betta splendens caught from thirteen provinces of central, northern, and western Thailand
(THNHM-F-01673, THNHM-F-01676 – 8, THNHM-F-01680 – 1, THNHM-F-01684, THNHM-F-01710, THNHM-F-01712 – 3, THNHM-F-01716, THNHM-F-01718, THNHM-F-01719, THNHM-F-01691 – 2, THNHM-F-01696, THNHM-F-01698 – 704, THNHM-F-01707 – 8, THNHM-F-01691 – 2).

Results

Betta siamorientalis sp. n.

Figs 1–3; Tables 1,2

Holotype. THNHM–F–01540 (36.1 mm SL), Thailand, Chachoengsao province, Bang Khla district, Tha Thonglang subdistrict, Sai Hai village (13°42′ N, 101°13′ E); *coll*. C. Kowasupat *et al.*, 12 Oct. 2010.

Paratypes. THNHM–F–01541, 10 ex., same locality as holotype; *coll*. C. Kowasupat *et al.*, 12 Oct. 2010. – THNHM-F-01836, 2 ex., same locality as holotype; *coll*. B. Panijpan *et al.*, 1 May 2012. – THNHM-F-01835, 1 ex., same locality as holotype; *coll*. C. Kowasupat *et al.*, 8 May 2012. – THNHM-F-01535, 3 ex., Thailand, Chachoengsao province, Bang Khla district, Sa Med Nuea subdistrict, behind central aquarist market (13°40′ N, 101°13′ E); C. Kowasupat *et al*. 6 Oct. 2010 – THNHM-F-01539, 12 ex., Thailand, Chachoengsao province, Bang Khla district, Sa Med Nuea subdistrict, behind central aquarist market (13°40′ N, 101°13′ E); T. Jeenthong *et al*. 12 Oct. 2010 – THNHM-F-01538, 10 ex., Thailand, Chachoengsao province, Bang Khla district, Sa Med Tai subdistrict, Nong Sano village, near border of Wang Klon village (13°38′ N, 101°11′ E); *coll*. T. Jeenthong *et al.* 12 Oct. 2010. –THNHM-F-01823, 5 ex., Thailand, Chachoengsao province, Bang Khla district, Sa Med Tai subdistrict, Wang Klon village (13°39′ N, 101°10′ E); *coll*. T. Jeenthong *et al.* 20 Dec. 2011. –THNHM-F-01831, 5 ex., Thailand, Chachoengsao province, Bang Khla district, Sa Med Tai subdistrict, Wang Klon village (13°39′ N, 101°10′ E); *coll*. T. Jeenthong *et al.* 8 May 2012. – THNHM-F-01824, 10 ex., Thailand, Chachoengsao province, Bang Khla district, Sa Med Tai subdistrict, Nong Sano village, Klum Ban Mor Taek (13°37′ N, 101°12′ E); coll. B. Panijpan *et al.* 26 Dec. 2011. – THNHM-F-01833, 2 ex., Thailand, Chachoengsao province, Bang Khla district, Sa Med Tai subdistrict, Nong Sano village (13°37′ N, 101°12′ E); coll. P. Ruenwongsa *et al.* 8 May 2012. – THNHM-F-01834, 1 ex., Thailand, Chachoengsao province, Bang Khla district (13°37′ N, 101°12′ E); *coll*. P. Ruenwongsa *et al.* 10 Jun. 2012. – THNHM-F-01825, 8 ex., Thailand, Chachoengsao province, Plaeng Yao district, Ban Bor subdistrict (13°35′ N, 101°15′ E); *coll*. P. Ruenwongsa *et al.* 3 Jan. 2012. – THNHM-F-01836, 1 ex., Thailand, Chachoengsao province, Phanom Sarakham district, Ko Khanun (Area 1) (13°42′ N, 101°26′ E); *coll*. A. Sakthawornlert 1 Oct. 2012. – THNHM-F-01837, 1 ex., Thailand, Chachoengsao province, Phanom Sarakham district, Ko Khanun (Area 2) (13°41′ N, 101°26′ E); *coll*. A. Sakthawornlert 1 Oct. 2012. – THNHM-F-01826, 5 ex., Thailand, Chon Buri province, Phanat Nikhom district, pond beside Kok Plor subdistrict administration building (13°34′ N, 101°8′ E); coll. C. Kowasupat *et al.* 10 Jan. 2012. – THNHM-F-01827, 3 ex., Thailand, Chon Buri province, Phanat Nikhom district, Kok Plor subdistrict, Noen Put village (13°30′ N, 101°6′ E); *coll*. C. Kowasupat *et al.* 15 Jan. 2012. – THNHM-F-01531, 2 ex., Thailand, Sa Kaeo province, Watthana Nakhon district, Chong Kum subdistrict, Chong Kum village (Area 1) (13°53′ N, 102°27′ E); coll. C. Kowasupat *et al.* 5 Oct. 2010. – THNHM-F-01532, 2 ex., Thailand, Sa Kaeo province, Watthana Nakhon district, Chong Kum subdistrict, Chong Kum village (Area 2) (13°53′ N, 102°27′ E); *coll*. C. Kowasupat *et al.* 5 Oct. 2010. – THNHM-F-01533, 2 ex., Thailand, Sa Kaeo province, Watthana Nakhon district, Chong Kum subdistrict, Chong Klum village (Area 3) (13°54′ N, 102°28′ E); *coll*. C. Kowasupat *et al.* 5 Oct. 2010. – THNHM-F-01534, 2 ex., Thailand, Sa Kaeo province, Watthana Nakhon district, Chong Kum subdistrict, Chong Klum village (Area 4) (13°54′ N, 102°28′ E); *coll*. T. Jeenthong *et al.* 5 Oct. 2010. – THNHM-F-01542, 3 ex., Thailand, Prachin Buri province, Kabin Buri district, Kabin Buri subdistrict, Tung Faek village (13°58′ N, 101°42′ E); *coll*. C. Kowasupat *et al.* 10 Dec. 2010. – THNHM-F-01543, 2 ex., Thailand, Prachin Buri province, Kabin Buri district, Hat Nang Kaeo subdistrict, Bueng Laem Hin (14°0′ N, 101°39′ E); *coll*. C. Kowasupat *et al.* 10 Dec. 2010. – THNHM-F-01544, 3 ex., Cambodia, Serei Saophoan, Banteay Meanchey province, Bod Tong village (13°39′ N, 102°45′ E); *coll*. B. Panijpan *et al.* 12 Jun. 2011.

Diagnosis. *Betta siamorientalis* sp. n. is distinguished from other members of the *B. splendens* group by having the dark-brown to black body; black opercle with two parallel reddish vertical bars; red patches on dark-brown to black opercular membrane; caudal fin rays with distal red crescent and thin black edge; absence of dark transverse bars on caudal fin; distal half of posterior anal fin rays red with a small red patch at distal tip; black and red from proximal pelvic fin base to the white tip.

Description. General body shape, appearance and colour pattern are illustrated in Figs. 1–3. Head small, short (head length 28.0–34.0 %SL), eyes large (orbit diameter 23.7–31.6 %HL); body slender (body depth at dorsal-fin origin 23.6–32.1 %SL); opercles parallel when head viewed dorsally; dorsal fin positioned after mid-body (predorsal length 57.9–67.1 %SL); caudal fin posterior margin rounded; dorsal fin distal margin rounded; pelvic fins falcate; anal fin pointed and most of male distal tip extended to half or more of caudal fin length (30 out of total 40). Meristic and morphometric data are summarised in Tables 1 and 2. Maximum known size is 32.7 mm SL (THNHM–F–01825).

Colouration. See Fig. 2 for colouration in live specimens. Male with dark-brown to black head, with iridescent yellowish-green to bluish-green scales; opercles with double parallel reddish vertical bars (a vertical bar on the edge of opercle redder than the inner one nearer to the base); opercular membrane dark-brown to black with red patches; eyes with iridescent yellowish-green to bluish-green patches at bottom and posterior regions; body background dark-brown to black with the iridescent yellowish-green to bluish-green scales: high ratio (greater than 60%) of body background (dark-brown to black) relative to scale iridescence (yellowish-green to bluish-green) in most specimens making it appear dark; dorsal fin rays dark-brown to black contrasting with iridescent yellowish-green to bluish-green interradial membranes, and at least proximal two-thirds having black transverse bars; caudal and anal fin rays red-brown to black contrasting with iridescent yellowish-green to bluish-green interradial membranes; caudal fin with distal red crescent and thin black edge; caudal fin interradial membranes with

Fig. 1. *Betta siamorientalis* sp. n., THNHM-F-01540, male, holotype, 36.1 mm SL, GenBank Accession Numbers: JQ818630 (COI), JQ815715 (ITS1).

Fig. 2. *Betta siamorientalis* sp. n., THNHM-F-01823, live adult male displaying aggression, paratype, 28.1 mm SL.

small black spots in some specimens; posterior anal fin with a small red patch at distal tip (some rays have red colour near the end, especially, those near the distal tip); anal fin interradial membranes with small black dots in some specimens; red-brown to black and red from proximal pelvic fin base to the white tip; pectoral fins hyaline. Female less colourful (see Fig. 3): head and body yellowish to light-brown with iridescent yellowish-green to bluish-green scales; opercles pale gold with two faint reddish vertical bars; throat with chin-bar; body with distinct upper central stripe nearly parallel to central stripe, at level of eye; caudal-peduncle black spot present; dorsal, caudal, anal, and pelvic fin rays yellowish with faint iridescent green to bluish-green interradial membranes; caudal fin with distal pale red crescent margin and pale thin black edge, or yellowish on both margin and edge; distal half of posterior anal fin with a small pale red or yellowish patch at distal tip; other characters similar to male.

Comparative notes. *Betta siamorientalis* sp. n. is distinguished from other members of the *B. splendens* group by having area belonging to the dark-brown to black body (background) greater than that of the iridescence of the scales (vs. red-brown to black body background in *B. splendens*; vs. area belonging to the dark-brown to black body (background) less than that of the iridescence of the scales in *B. imbellis*); black opercles with two parallel reddish vertical bars (vs. double iridescent green to bluish-green vertical bars in *B. imbellis* and *B. mahachaiensis*; vs. opercle with iridescent green scales in *B. smaragdina* and *B. stiktos*); red patches on brown-to-black opercular membrane (vs. absence in *B. mahachaiensis*); caudal

Fig. 3. (**a**) *Betta siamorientalis* sp. n., THNHM-F-01824, live male (acclimatized), paratype, 30.9 mm SL; (**b**) *Betta siamorientalis* sp. n., THNHM-F-01824, live female (acclimatized), paratype, 26.7 mm SL.

fin with distal red crescent and thin black edge (vs. absence in *B. smaragdina*, *B. stiktos*, and *B. mahachaiensis*); absence of dark transverse bars on caudal fin (vs. presence in *B. stiktos*); posterior anal fin rays red with a small red patch at distal tip (vs. absence in *B. smaragdina*, *B. stiktos*, and *B. mahachaiensis*); red-brown to black and red from proximal pelvic fin base to the white tip (vs. absence in *B. stiktos*, and *B. mahachaiensis*); absence of iridescent bluish-green front margin of pelvic fin (vs. presence in *B. stiktos*, and *B. mahachaiensis*).

Distribution. Based on our extensive survey of fighting fish in sixty-seven (total of seventy-seven as of 2012) provinces, the wild *B. siamorientalis* sp. n. is currently found in only 4 provinces in Thailand: Chachoengsao, Sa Kaeo, Prachin Buri, and Chon Buri (only in areas close to Chachoengsao). The eastern region of Thailand harbouring this species is shown in Fig. 4. *Betta siamorientalis* sp. n. is also found in Banteay Meanchey, a province of Cambodia on the eastern border of Sa Kaeo province: this may indicate an even wider and farther distribution. It is worth mentioning that in the Chon Buri province, *B. siamorientalis* sp. n. has been found only in Phanat Nikhom district which is next to Chachoengsao province, while in the eastern part of Chon Buri, that is connected to other provinces, only *B. splendens* has been found.

Field notes. *Betta siamorientalis* sp. n. was found in the shallow freshwater marshes, grass fields, and paddy fields. It was also caught at the water edge (with dense vegetation) of ponds, lagoons, ditches, canals, and others water ways. *Leersia hexandra* (family

Table 1. Morphometric data of *Betta siamorientalis* sp. n., *Betta imbellis*, and *Betta splendens*.

SPECIES	*Betta siamorientalis* sp. n.				*Betta imbellis*			*Betta splendens*		
	Holotype	Paratype								
Number of specimens	1	40			40			40		
		Min.–Max.	Mean	S.D. (±)	Min.–Max.	Mean	S.D. (±)	Min.–Max.	Mean	S.D. (±)
Standard length (mm)	26.6	22.5–32.7	28.6	2.2	21.2–33.8	27.7	4.2	19.9–35.0	28.0	4.2
(% SL)										
Total length*	135.7	126.5–145.5	133.3	4.4	125.1–142.5	133.3	3.6	123.8–139.1	132.2	3.8
Predorsal length	59.4	57.9–67.1	63.0	1.8	59.1–68.1	63.2	2.5	57.1–68.2	62.9	2.4
Postdorsal length	26.6	19.3–26.7	22.3	2.0	17.7–27.9	22.0	2.8	19.1–27.2	22.6	2.1
Preanal length*	34.1	35.0–42.7	38.8	2.2	35.0–43.0	38.5	2.4	32.1–44.5	37.4	2.8
Body depth	27.6	23.6–32.1	27.7	2.0	23.4–30.2	26.7	1.9	22.9–31.3	26.6	2.0
Caudal peduncle depth	19.1	14.8–21.8	18.4[a]	1.2	15.3–22.8	18.5	1.6	13.9–21.3	17.2[a]	1.7
Dorsal fin base length	17.7	12.8–20.6	16.9	2.2	14.7–21.1	17.5[b]	1.6	12.1–19.3	15.8[b]	1.7
Anal fin base length	64.4	57.6–70.8	63.8	3.2	57.1–68.4	61.4	2.8	53.3–70.1	61.9	3.9
Pectoral fin length	21.6	15.0–24.4	19.1	1.8	16.6–21.9	19.6	1.4	15.3–24.4	19.4	2.0
Pelvic fin length	43.1	20.2–58.2	38.5	8.6	26.8–52.2	39.3	5.9	21.2–55.0	35.5	8.0
Head length*	30.2	28.0–34.0	30.0	1.4	26.6–31.5	30.0	1.2	27.7–33.1	30.4	1.4
(% HL)										
Snout length	19.8	18.6–30.0	23.9	2.3	18.5–28.4	22.9	2.1	18.1–28.6	23.1	2.3
Orbit diameter	28.0	23.7–31.6	27.7	1.9	24.4–31.0	27.7	1.8	23.9–28.9	26.5	1.4
Interorbital length*	9.3	7.8–14.2	11.1	1.5	8.3–13.5	10.7	1.3	8.6–13.7	10.6	1.5
Postorbital length	47.2	48.3–56.8	51.5	2.3	45.8–57.7	51.8	2.9	46.1–55.3	50.4	2.3

* Nonnormal distribution by using the Shapiro-Wilk test for normality ($\alpha = 0.05$)

[a] *B. siamorientalis* sp. n. is significantly different from *B. splendens* ($\alpha = 0.05$, Bonferroni-adjusted)

[b] *B. imbellis* is significantly different from *B. splendens* ($\alpha = 0.05$, Bonferroni-adjusted)

Table 2. Meristic data of *Betta siamorientalis* sp. n., *Betta imbellis,* and *Betta splendens*.

SPECIES	*Betta siamorientalis* sp. n.			*Betta imbellis*		*Betta splendens*	
	Holotype	Paratype					
Number of specimens	1	40		40		40	
		Min.–Max.	Mode	Min.–Max.	Mode	Min.–Max.	Mode
Subdorsal scales	7	5–7	6	5–8	6	5–7	6
Transverse scales	9	8–9	9	8–9	9	8–9	9
Lateral scales	30	28.5–31.5	30	28.5–31.5	30	28.5–32	30
Predorsal scales	23	20–26	23	21–26	23	20–27	24
Postdorsal scales	8	8–11	9	8–12	9	8–12	10
Lateral scales below dorsal-fin origin	15	14–16	15	14–16	16	14–17	16
Lateral scales below anal-fin origin	5	5–8	7	5–8	7	4–7	6
Anal fin rays (spines)	4	3–5	4	3–5	4	3–5	4
Anal fin rays (articulated)	23	21–25	23	21–26	22	22–26	23
Dorsal fin rays (spines)	1	1–2	1	1–2	1	1–2	1
Dorsal fin rays (articulated)	9	7–11	9	7–10	9	7–10	9
Pectoral fin rays	14	10–14	12	11–14	12	11–14	13

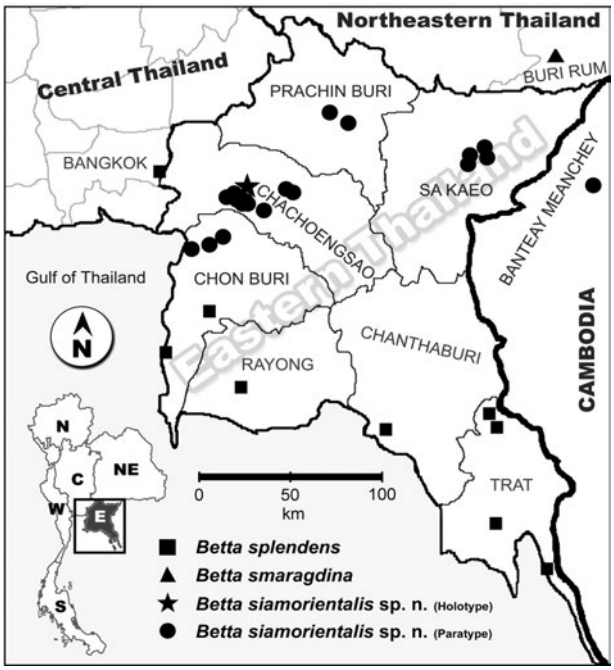

Fig. 4. Map of eastern provinces of Thailand showing known distribution of *B. siamorientalis* sp. n. (star: holotype, circle: paratype), *B. splendens* (square), *B. smaragdina* (triangle).

Poaceae) and *Eleocharis dulcis* (family Cyperaceae) were common in such habitats. The habitat characteristics are quite similar to those of other members of *B. splendens* group (see Fig. 5). *Betta siamorientalis* sp. n. lives in still waters and uses water plants as shelter to set up safe territory for building the bubble-nest, courtship, and hatching of fry, the same preferences as other species of the *B. splendens* group. Other labyrinth fishes cohabiting with *B. siamorientalis* sp. n. are *Trichopsis vittata*, *Trichopsis shalleri*, *Trichopsis pumila*, *Trichopodus trichopterus*, *Anabas testudineus*. Other non-labyrinth fishes sharing the same habitats are *Lepidocephalichthys hasselti*, *Pangio anguillaris*, *Macrognathus siamensis*, and *Monopterus albus*.

Etymology. The name siamorientalis is adopted as an allusion to the type locality of the new species collected in this study. It is derived from Siam, the former name of Thailand, and orientalis, a Latin version for east, the region of the country where the fish is located.

Remarks. There is only one indigenous species in the catch sites. It should be noted that although most specimens of *B. siamorientalis* sp. n. have reddish vertical bars on the black opercle, the bar colour can vary, for example, reddish to pale red, greenish-silvery or just silvery or no colour in some populations. That *B. siamorientalis* sp. n. is a new species and not a hybrid is supported by the results from DNA barcoding using a mitochondrial gene (cytochrome c oxidase I or COI) and a the nuclear gene (internal transcribed spacer 1 or ITS1) along with a phylogenetic tree (in preparation). The results reveal that *B. siamorientalis* sp. n. is closer to *B. imbellis* than *B. splendens* and other congeners. Our accession numbers (www.ncbi.nlm.nih.gov) of the COI gene and ITS1 gene of the holotype *B. siamorientalis* sp. n. are JQ818630 (COI) and JQ815715 (ITS1). For those of the *B. siamorientalis* sp. n. paratypes and of the *B. imbellis* and *B. splendens* see for example; JQ818699–706 and JQ818708–23 for COI gene of *B. siamorientalis* sp. n. and JQ818620–36 for ITS1 of *B. siamorientalis* sp. n.; JQ818776–81, JQ818783, and JQ818786–96 for COI gene of *B. imbellis*; and JQ818594–605, JQ818607, and JQ818610–19 for ITS1 of *B. imbellis*; JQ818724–728, JQ818784–5, and JQ818797–806 for COI gene of *B. splendens*; and JQ818637–40, and JQ818608–9 for ITS1 gene of *B. splendens* in gene bank website (www.ncbi.nlm.nih.gov).

Discussion

Betta siamorientalis sp. n. shares common features with other congeneric species in the *B. splendens* group (see Witte & Schmidt (1992); Tan & Ng, 2005). However, upon close scrutiny of more than one hundred specimens from the four provinces of eastern Thailand and one province in Cambodia (next to the eastern border of Thailand), *B. siamorientalis* sp. n. can be well distinguished from other known members of the group: it has a slender body with a rather blackish colour due to a smaller proportion of area on the body for iridescent blue/green scales relative to the black body background. It differs from *B. imbellis* and *B. smaragdina* in having reddish bars on the black opercle vs. green to bluish-green bars of the former and iridescent green scales on opercle of the latter. Nevertheless, it is rather difficult to differentiate between the less acclimatized male and female members of *B. siamorientalis* sp. n. due to the pale colour of the body. Most *B. siamorientalis* have a light reddish colour on the opercle edge, which may lead some people to mis-identify them as *B. splendens*. Indeed, this new species has previously been described as *B. splendens* by Lertpanich & Aranyavalai (2007), perhaps because of the authors′ overreliance on the red parallel bars on the opercle as a criterion while disregarding the body and fins which are similar to those of *B. imbellis*. On the other hand, Kühne (2008) named the so-called black imbellis from Cambodia as *B.* cf. *imbellis* probably due to its general body shape and fin colours similar to those of *B. imbellis*. Similarly, Schäfer (2009) called his Vietnam black betta, *B.* cf. *imbellis*. We realize that there may be others who have

Fig. 5. Habitats of *B. siamorientalis* sp. n. in eastern provinces of Thailand: (**a**) Chachoengsao (type locality) (12 Oct. 2010); (**b**) Chon Buri (10 Jan. 2012), (**c**) Sa Kaeo (5 Oct. 2010), (**d**) Prachin Buri (10 Dec. 2010).

also noticed the distinct characters of this fish, including TEERAWAT UTAPONG (personal communication) who has remarked that the fish from Kabin Buri district (Prachin Buri province) could possibly be quite different from *B. splendens*. As an important step toward distinguishing the black imbellis fish from the others, our extensive survey covering all eastern provinces of Thailand (including those on the Thai border shared

with Cambodia), yields morphological results which show *B. siamorientalis* sp. n. to be a new species member of the *B. splendens* group. Even though the morphometric and meristic data seem to show only slight differences in some of these characters (Tables 1, 2), our statistical analysis, nevertheless, reveals a significantly higher caudal peduncle depth in *B. siamorientalis* sp. n. when compared to that of *B. splendens*. The lack of clear differences in morphometric and meristic characters is consistent with Tan & Ng (2005) statement that it is rather difficult to use meristic characteristics and morphometric data to differentiate species within members of the same group due to the rather conservative nature of these characters (see also Tan & Tan, 1996). The overall results, however, suggest that *B. siamorientalis* sp. n. is more closely related to *B. imbellis* than *B. splendens* despite the red vertical bars on the opercle. This is further supported by our DNA barcoding results (in preparation).

Based on our survey, the distribution of *B. siamorientalis* sp. n. in Thailand is apparently restricted to certain areas in the upper eastern region, namely, Chachoengsao, Chon Buri (only in areas bordering Chachoengsao), Prachin Buri, Sa Kaeo. The fish is also found at Banteay Meanchey, a Cambodian province near Sa Kaeo. We have not found representatives of this group as yet in lower eastern provinces namely Rayong, Chantaburi, and Trat, as well as Koh Kong of Cambodia (close to Trat province of Thailand). Nevertheless, the bubble-nest builder *B. splendens* is generally found in these latter areas. Recent reports on the findings of the fish from Cambodia (Kühne, 2008; Linke, 2009) and Vietnam (Schäfer, 2009) suggest that at present the fish may be inhabiting certain regions of eastern Thailand through parts of Cambodia that connect the southern region of Vietnam. However, *B. imbellis*, which is closely similar to *B. siamorientalis* sp. n., is found in the southernmost provinces of Peninsular Thailand through to the upper part of peninsular Malaysia. In an attempt to explain the similarities, palaeogeographical evidence is called upon here. During the most recent ice age with lowered sea level, the land of southern peninsular Thailand is believed to have connected via a land mass, together with a major river system(s), to the land of the eastern Thailand, southern Cambodia, and Vietnam. Due to the rise of sea level after the ice age, the land mass is currently under water in the Gulf of Thailand thus separating the two land areas (Dodson *et al*., 1995; Voris, 2000; Sathiamurthy & Voris, 2006). This geographical evidence may at least partially explain that the same or very similar fish in the two places, separated by an expanse of sea water since the big thaw, still retain similarities; there are some visible differences due to the time that has elapsed since then for the divergence (allopatric speciation) in their new environments. In addition to the bubble-nest builders above, the mouth-brooder in eastern Thailand, *B. prima* (Kottelat, 1994) and the southern brooder *B. pallida* (Schindler & Schmidt, 2004) in the southern peninsula also share similar characters. It is thus plausible that *B. siamorientalis* sp. n. and *B. imbellis* have evolved from a common ancestor(s). However, we cannot explain the fact that no mouth-brooders have been found in the upper part of the Thai pennisula from Petchaburi to upper Chumporn in spite of its present connectivity to the more extreme southern areas (south of Chumporn province) where mouth-brooders are common.

Regarding the phylogenetic relationship between congeners, previous results from DNA barcoding of bubble-nest builder bettas using cytochrome c oxidase (COI) and 16S mitochondrial gene (Sriwattanarothai *et al.*, 2010) showed that the species in the *B. splendens* group are well separated phylogenetically into at least four main branches of *B. splendens*, *B. imbellis*, *B. mahachaiensis*, and *B. smaragdina*. *Betta stiktos*'s COI barcoding has now been done and shown to be different and yet very close to that of one type of *B. smaragdina* found in the Northeastern part of Thailand (in preparation). Our preliminary results based on both COI mitochondrial gene and internal transcribed spacer 1 (ITS1) nuclear gene point to *B. siamorientalis* sp. n. as well separated phylogenetically from the *B. imbellis*, albeit closely related to the latter than *B. splendens*. It is well distinct from *B. splendens* and other congeners. Our DNA barcoding results (in preparation) also show that *B. siamorientalis* sp. n. have distinctly different maternal lines from other congeners: of the numerous *B. siamorientalis* sp. n. specimens studied so far none has the COI sequence identical or nearly identical to those of members of the *B. splendens* nor *B. imbellis* (see accession number of COI and ITS 1 gene in Remarks section). The results thus indicate that *B. siamorientalis* sp. n. is not a hybrid between *B. imbellis* and *B. splendens*. Another result from our barcoding work is that, despite opercular differences among specimens of the *B. siamorientalis* sp. n., all the above nuclear and mitochondrial DNA sequences remain remarkably identical and they all belong to this same species. Such opercular variation in terms of presence or absence of red bars is also observed in the large numbers of *B. splendens* studied by us.

At this juncture, we wish to address the value of the above short DNA sequences in complementing other characters for species identification. The COI DNA for barcoding or other short DNA sequences as tools for species differentiation and identification, have proved to be useful (Hebert *et al.*, 2003), albeit not perfect in this role (Krishnamurthy & Francis, 2012). Nevertheless these sequences are not just of any short pieces of DNA but ones judiciously selected for the purpose (Ratnasingham & Hebert, 2007). Some re-

cent publications (Dasmahapatra *et al.*, 2010) may emphasize certain deficiencies in the barcoding methodology and the latter may not be the sole DNA-based species distinguishing tool for the future (Taylor & Harris, 2012), it is still helpful in complementing morphological and other criteria. For example, the various commonly bred and traded betta fighting fish obtained by us in Thailand, be it as ornamentals or fighters, show vast differences in terms of colour, shape, fin, size, aggressiveness, etc., and yet the COI evidence indicates that they all belong to the same *B. splendens* wild type′s profile. This is consistent with the fact that breeders have used *B. splendens* mainly for selective breeding. Without the prior knowledge of how this vast variety of ornamentals and bred fighters have come about, these living things might be thought to be different species; and yet they prove to be nearly identical by DNA barcoding.

Here, we have another situation where *B. siamorientalis* sp. n. and *B. imbellis* appearing not much different based on morphometric, meristic and other external criteria thus making distinction between the two difficult. We therefore have to resort to the DNA evidence to further help us to decide whether the two are the same or different. From the COI barcoding (mitochondrial DNA) and ITS1 (nuclear DNA) data we are confident the two are different enough to be called different species. (The nuclear DNA can be derived from both maternal and parental lines, so it can show up a hybrid, which is not found here.) At present, *B. imbellis* and the black imbellis are known to locate very far apart geographically. Without any prior knowledge of their distant catch sites, even experienced persons presented with the two fish for the first time might perceive them to be identical or nearly so. Some might even mistake the black imbellis to be *B. splendens*. As mentioned above, there is a situation similar to the above in that in appearance *B. stiktos* and one type of *B. smaragdina* (type locality) appear very similar. We suggest that these two lines of complementary evidence above should be applied to determine whether *B. stiktos* is different enough to the nearest type locality *B. smaragdina* to be called a different species just as in the case of *B. imbellis* and *B. siamorientalis* sp. n.

At present, many people including breeders and catchers still believe that all native fighting fish in the aforementioned four provinces to be *B. splendens*. People in the villages say that since their ancestral times the fish has always been in this region, albeit not used for fighting in the gambling den because as the word imbellis implies, it is not very fierce. We have never heard any story about southern *B. imbellis* brought to the eastern part for either fighting or cross-breeding. We know enough about the local fish fighting scenes and practices not to believe that *B. siamorientalis* sp. n. are the descendants of the discarded *B. imbellis* (brought from the south) in recent past. When the new species status of *B. siamorientalis* sp. n. has been formally accepted, this knowledge will be disseminated for conservation and protection of this species.

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